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Special Section:
Biogeochemistry of Natural Organic Matter

Key Points:
- Tea Bag Index (TBI) field incubation experiments should span a period of >1 year to ensure that all stages of early organic matter (OM) decomposition are observed
- Soil characteristics are a major control on long-term OM decomposition in intertidal environments
- The TBI should be used with caution in intertidal environments and is not a universal proxy for organic carbon (OC) degradation

Supporting Information:
- Supporting Information S1

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An Assessment of the Tea Bag Index Method as a Proxy for Organic Matter Decomposition in Intertidal Environments

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Abstract Intertidal wetlands capture and store carbon (C) for long periods of time, helping to reduce the concentration of CO_2 in the atmosphere. Yet the processes, which govern the decomposition and subsequent long-term storage of organic matter (OM) and C in these habitats, remain poorly understood. The Tea Bag Index (TBI) uses a standardized OM (green and Rooibos tea) and has the potential to shed light on OM decomposition across habitats, including saltmarshes. Here, we apply the TBI method at two saltmarshes within the same estuary with the aim of (i) reducing the influence of climatic variables and (ii) determining the role of the environment, including the soil characteristics, in the decomposition of OM. We extended the standard (3 months) incubation period over a full year in order to investigate the longer-term decomposition processes at each site. The initial results partially support previous studies that the early stages of decomposition (leaching of the water-soluble fraction) is governed by climatic conditions, but may be further enhanced by tidal flushing in saltmarshes. By extending the incubation period, we observed the initiation of midstage OM decomposition (cellulose degradation) upon which the soil characteristics appear to be the dominant control. These results highlight the importance of long-term TBI incubations to understand early-stage OM decomposition. The relationship between tea mass (OM) loss and C loss in these intertidal environments is not straightforward, and we would caution the use of the TBI as a direct universal proxy for soil C degradation in such intertidal wetlands.

Plain Language Summary Saltmarshes capture and lock organic matter (OM) and carbon (C) away in their underlying soils, preventing some of that C being released back to the atmosphere. Yet how this OM and C breaks down within these environments is poorly understood. The Tea Bag Index (TBI) uses standardized OM in the form of green and Rooibos tea to investigate decomposition. Burying the tea for different time periods allowed us to measure the mass and C loss to understand the breakdown of the material over time. Burying the tea bags in two different marshes within the same estuary allowed us to focus on the soil characteristics rather than climatic conditions. The yearlong incubations showed that the early breakdown of the OM is likely controlled by the climate and tidal flushing of the marshes, but that the second phase of decomposition is governed by the soil characteristics. The TBI is being used as a surrogate for the breakdown of soil C we tested, if mass loss of the tea relates to C degradation. The results suggest that the TBI is currently a poor proxy for C degradation in saltmarshes and that the TBI in these environments should be treated with caution.

1. Introduction

Intertidal wetlands, including saltmarshes, sequester carbon at significantly higher rates than many globally significant habitats such as tropical rainforests, making them one of the most important carbon (C) sinks on Earth (Macreadie et al., 2013; Wylie et al., 2016). This C sequestration capacity is attributed not only to the effective conversion of carbon dioxide (CO_2) into plant biomass C, but also to the efficient capture of allochthonous (transported) and autochthonous (in situ) organic carbon (OC; Chmura, 2013; Kelleway et al., 2017). Saltmarsh vegetation traps allochthonous OC during tidal flooding and accumulates autochthonous C through burial of in situ plant litter and roots (Howard et al., 2014; Kirwan & Megonigal, 2013). In conjunction with tidal inundation, low oxygen sediment conditions prevail, slowing decomposition such that recalcitrant (resistant to decomposition) C may remain in saltmarsh stores for millennia (Elsey-Quirk et al., 2011; Kelleway et al., 2017). Clearly, these intertidal environments are globally important sites for C sequestration and storage. However, there is a general lack of empirical data on saltmarsh C dynamics, notably regarding the environmental factors controlling in situ decomposition of organic matter (OM; Mueller et al., 2016; Janousek et al., 2017; Stagg et al., 2018). As C stored in saltmarshes can be autochthonous, understanding the rates and drivers of litter decomposition is essential to both manage these ecosystems and model how climate change may affect their C storage (Middleton & McKee, 2001).

Past studies have monitored the decomposition of litterbags within ecosystems to determine the drivers of litter decomposition and estimate C storage efficiency (Alsafran et al., 2017; Benner et al., 1991; Didion et al., 2016; Djukic et al., 2018; Enríquez et al., 1993; Prescott, 2010). However, variations in the quantity and composition of the leaf litter used make intersite comparisons challenging (Djukic et al., 2018; Tiegs et al., 2007). The Tea Bag Index (TBI), developed by Keuskamp et al. (2013), uses individual commercially available tea bags in place of leaf litter to overcome the inconsistencies of traditional litter bags while retaining many of the characteristics of native litter decomposition dynamics (Didion et al., 2016). There are only a few published examples of the TBI used in coastal habitats, with very few focusing solely on saltmarsh habitats. Studies attempting to fill this research gap have focused on the relationships between decomposition and climate, sea level, and vegetation cover (Alsafran et al., 2017; Djukic et al., 2018; Mueller et al., 2018). Although the TBI has been described as a potential method to understand C loss and storage (Djukic et al., 2018), it does not directly measure C degradation; rather, it uses OM mass loss as a qualitative proxy for C loss, a relationship which has not been tested in saltmarsh environments. An improved understanding of how OM decomposition relates to OC loss is therefore highly desirable in the consideration of the TBI as a proxy for OC degradation within complex saltmarsh environments.

Soil characteristics and other local environmental parameters are largely overlooked in TBI studies but are probably significant in governing in situ litter decomposition and C storage. Here we use the TBI to examine the influence of different soil characteristics on litter decomposition at two tidal saltmarshes in Belhaven Bay, Scotland. We also expand upon the previously published uses of the TBI (Djukic et al., 2018; Mueller et al., 2018) by extending the in situ incubation time to a year and by exploring how the decomposition of OM relates to the loss of OC. An improved understanding of early OC degradation in saltmarsh environments will provide new insights into the processes governing long-term C storage in coastal wetlands.

2. Study Site

The study area is located on the east coast of Scotland stretching from Belhaven to Tyninghame (Figure 1). The area comprises two saltmarsh types located approximately 1.7 km apart and separated by an intertidal flat within the Tyne estuary. In this study, we focus on the estuarine and adjacent intertidal flat (Figure 1d) and a back-barrier marsh (Figure 1e).

The estuarine marsh occupies an area of 0.06 km^2 and is characterized by intertidal C3 vegetation formed on raised mud platforms intersected by a series of creeks. The marsh at Belhaven backs onto an embankment built between 1832 and 1853 which allowed part of the marsh to be reclaimed for agriculture (Figures S3–S5 in the supporting information). In comparison to the back-barrier marsh, the estuarine site has a greater diversity and biomass of vegetation dominated by *Salicornia europaea, Puccinellia maritima*, and *Plantago maritima* species. The underlying saltmarsh peat ranges in thickness from ~10–40 cm throughout the marsh.

Back-barrier intertidal marshes are relatively rare in Scotland, with the Tyne marsh representing one of the largest (0.56 km^2) in the country (Haynes, 2016). Examination of historic maps (Figures S1 and S2) suggest that the back-barrier marsh only recently evolved, likely as a result of the North Sea storm of 1953 (Swindles et al., 2018). The marsh's relatively young age is reflected in both the low diversity of C3 vegetation and poorly developed thin saltmarsh peat. The back-barrier soil, in places, is characterized by a thin layer of saltmarsh soils (2–5 cm) overlying sand. The uppermost layers of sand are characterized by a black color, likely due to sulfide reduction and associated low oxygen conditions (Howarth, 1984) ideal for OM preservation.

The region is characterized by a temperate climate with a mean annual temperature (MAT) of 9.5 °C and mean annual precipitation (MAP) of <700 mm; full climate records for the duration of the study can be found in the supplementary material. By using these colocated yet contrasting saltmarshes to investigate the degradation and preservation of OM, we largely negate many major environmental variables such as

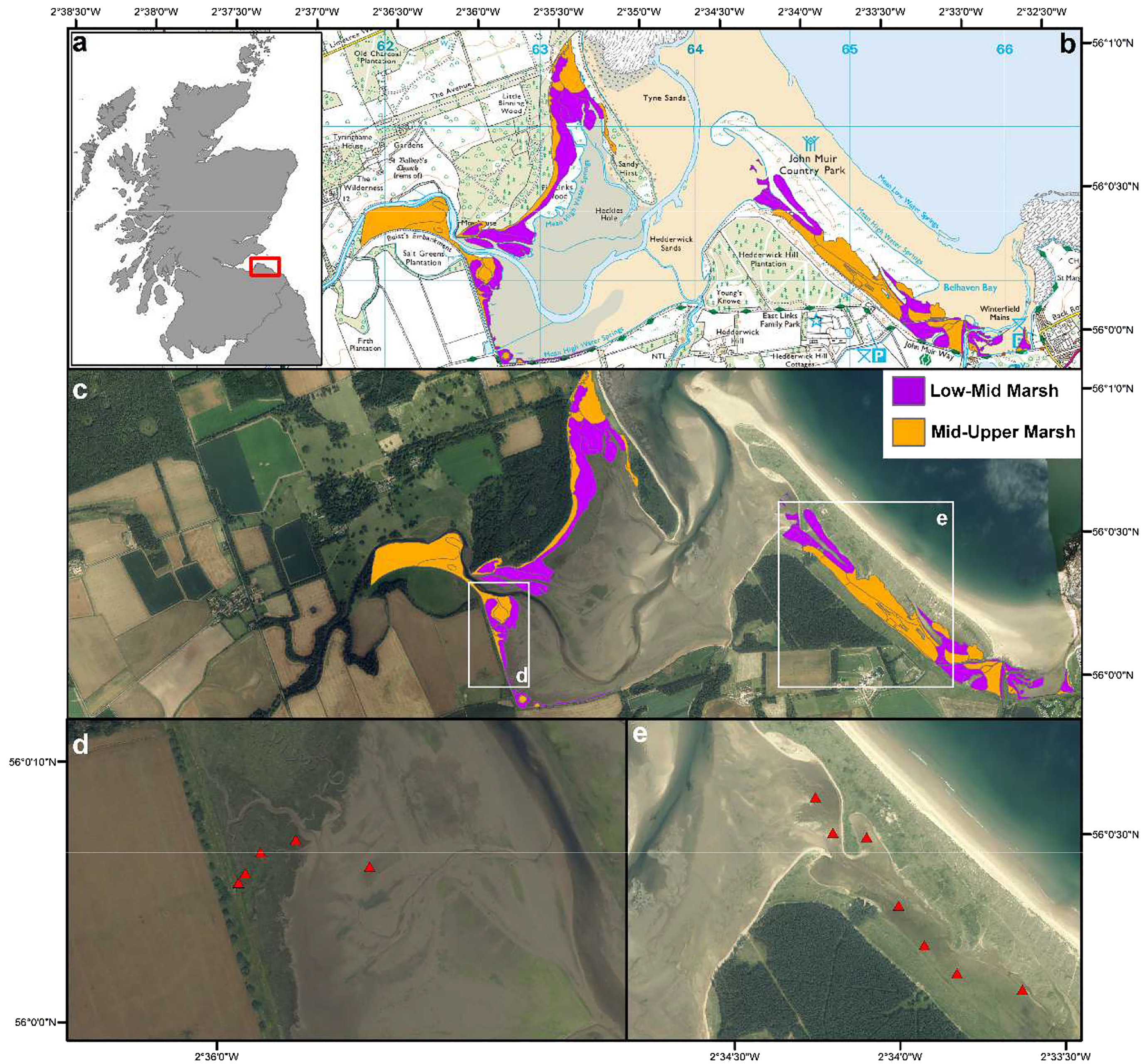

Figure 1. Location map detailing (a) the site in context of Scotland and (b) Ordnance Survey Map detailing the location of the Tyne estuary in relation to Dunbar with the high and low marsh highlighted (Haynes, 2016). (c) A regional overview. Highlighted are the teabag burial locations (▲) across the different marsh environments. (d) Estuarine and (e) back-barrier. © Crown copyright and database rights [2018] Ordinance Survey (100025252).

climate and tidal inundation (Sup Fig S6-7), therefore allowing a greater focus on the potential influence of the physical properties of the sediment.

3. Materials and Methods

3.1. Tea Bags

Following the method proposed by Keuskamp et al. (2013), we used both green tea and Rooibos tea bags as standardized materials for organic plant material decomposition. Green tea is a more labile material with expected faster decomposition, and Rooibos tea is a more recalcitrant material with expected slower rates of decomposition (Keuskamp et al., 2013). Each tea bag was made of woven nylon, ensuring the bag itself would not decompose, and individually weighed; the tea bags contained 2.17 ± 0.12 and 1.93 ± 0.35 g of green and Rooibos tea, respectively. The small mesh size of ~0.25 mm allows access of microfauna and fine plant rootlets, but inhibits macrofauna from entering (Bradford et al., 2002).

We used the linear method as outlined by Howard et al. (2014) from the dominant shoreline of each marsh and working inland. This sampling method captures the different intertidal ecological communities and potentially the variability in OM decomposition, which generally form perpendicular to the shoreline (Howard et al., 2014). In the estuarine marsh the teabags were buried below the root-mat at a depth of 10 cm following the standard methodology (Djukic et al., 2018; Keuskamp et al., 2013). In comparison, the shallow poorly developed soils of the back barrier marsh required the teabags to be buried at a depth of 2–5 cm to remain below the root-mat but still in the organic layer. In June 2017, we buried 12 tea bags of each type at each site and recorded their locations using differential GPS.

We subsequently collected four teabags at 100 days (*Collection 1*), 266 days (*Collection 2*), and 329 days (*Collection 3*). We were unable to recover the teabags from some sites because of tidal inundation at the time of collection. Three additional teabags of each type were buried at all sites during Collection 2 to compare decomposition of the OM to that recorded from Collection 1 and to study seasonal variations; these teabags were incubated for 63 days (*Collection 4*).

3.1.1. Tea Characterization

Unlike Keuskamp et al. (2013), this study uses locally sourced tea (tlc: St Andrews, www.drinktlc.com) instead of the Lipton® brand, so we undertook several experiments to compare both the similarity of different tea brands and the suitability of tea to this experiment. To determine the composition of the two tea types, we used elemental analysis to quantify the C, nitrogen (N), and C/N ratio. We dried the tea at 40 °C for 24 hr, milled it to a powder, and then placed a 20mg subsample in a tin capsule for analysis using an Elementar EL Vario at the University of St Andrews following the methodology of Verardo et al. (1990). C/N ratios were reported as molar ratios where C/N = (OC/12)/(TN/14). We estimated the analytical precision for the entire study by repeat analysis of standard reference material B2178 (Medium Organic Content Standard; Elemental Microanalysis, UK) with C = 0.08% and N = 0.02%. Additionally, thermogravimetric analysis (TGA) was undertaken on both the tlc: St Andrews and Lipton® teas allowing further comparisons of the two brands of tea. TGA analysis was undertaken using a Mettler Toledo TGA2 at the University of St Andrews, where ~20 mg was heated from 40 to a 1000 °C at a rate of 10 °C/min within a stream of N_2.

To quantify both the water-soluble fraction and its loss rate, we used a simple incubation experiment, where both teabag types ($n = 10$) were placed in total organic carbon- (TOC)-free glassware and submerged in 150 ml of TOC-free water for 12 hr at 10 °C to replicate the mean temperature of the region and tidal inundation. After 12 hr the teabags were dried and weighed and the incubation was repeated with fresh water until no further mass loss was observed. At the end of the experiment, the above analytical methodology was used to measure the elemental composition (C and N) of the tea.

To determine the mineral fraction of the tea, loss on ignition was undertaken (Dean, 1974), where 1 g of the remaining tea material (leached) was combusted at 550 °C for 8 hr. The material remaining after the combustion (i.e., ash) is considered to be the mineral fraction. Unlike Keuskamp et al. (2013), we did not carry out a chemical extraction to determine the NPE: nonpolar extractable (fats and waxes), ASF: acid soluble (cellulose), and AIF: acid insoluble (lignin) fractions; rather, we subtracted the water soluble and mineral fraction from the original tea mass to estimate the “extractable fraction” of the tea.

Because this approach to characterize the tea differs from the chemical extraction methodology used by Keuskamp et al. (2013), we undertook a comparative study using the Lipton® brand used in the original TBI studies (Djukic et al., 2018; Keuskamp et al., 2013). The tea was provided to this study through the TeaComposition H_2O project. The Lipton® brand teas were characterized using the same leaching and thermal processes used in this study, thereby allowing the results from this study to be directly compared to the wider TBI literature.

3.2. Soil Characterization

To characterize the saltmarsh substrate and OM preservation conditions, soil samples were collected at the teabag burial depth from each site for geochemical and physical property analysis. We recorded the soil profile and removed a subsample from the teabag burial depth for geochemical and physical property analysis.

3.3. Postcollection Analysis

3.3.1. Tea

The teabags were collected from the field, gently cleaned of soil and roots using deionized (TOC-free) water and dried in an oven at 40 °C for at least 48 hr (Keuskamp et al., 2013). We then cut open the tea bags, weighed their contents, and removed any visible roots. The weight of the incubated tea bags themselves were not recorded, because the bags were often damaged with dissolved tags or missing strings (Djukic et al., 2018). In addition to recording the mass of the incubated tea, we also measured its elemental composition following the methodology detailed above (section 3.1.1).

3.3.2. Soil Samples

To characterize the soil, the subsampled cores underwent both physical property and geochemical analysis. We recorded the samples' wet and dry masses following oven drying and calculated the dry bulk densities of the sediment following Howard et al. (2014). The dry samples were subdivided for particle size and geochemical analysis. Particle size analysis was undertaken following (Blott et al., 2004), where the organic and carbonate fractions are removed by hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) treatments, respectively. Finally, sodium hexametaphosphate was added to disperse samples before using a Beckman Coulter LS230 to measure the particle size (<2 mm) of the reaming minerogenic fraction (Austin & Evans, 2000).

We milled the other half of the dried subsamples to a powder and placed ~20 mg of material into both tin and silver capsules. The tin capsules were analyzed following the methodology outlined above (section 3.1.1) producing total carbon and N data. The soil within the silver capsules were treated with HCl to remove carbonate (Harris et al., 2001) and after drying (24 hr at 40 °C) the capsules underwent elemental analysis to quantify the OC content (Harris et al., 2001). We calculated the quantity of inorganic carbon in each sample by subtracting the OC from total carbon.

To determine stable isotope values, we weighed out ~12 mg of milled sediment into silver capsules. The samples in silver capsules underwent an acid fumigation step (Harris et al., 2001) to remove carbonate. After drying for 24 hr at 40 °C, we measured the $\delta^{13}C_{org}$ using an elemental analyzer coupled to an isotope ratio mass spectrometer at OEA Labs and reported the values in standard delta notation relative to Vienna Peedee belemnite. Analytical precision was estimated from repeat analysis ($n = 6$) of standard reference material USGS40 (*L-Glutamic Acid*). The precision for $\delta^{13}C$ was 0.07‰.

4. Results and Interpretation

4.1. Teabag Characterization

The physical and chemical characteristics of the green and Rooibos tea used in this study are largely comparable to the Lipton® tea used by Keuskamp et al. (2013; Table 1), this is further supported by the thermograms produced from the TGA analysis (Figure 2) which clearly illustrate the compositional similarity of the tlc: St Andrews tea to that of the Lipton® brand used by Keuskamp et al. (2013).

While this study uses different characterization approaches to understand the composition of the tea, the comparative experiment suggests minimal differences between the methods (Figure S8 in the supporting information). When the leaching and thermal treatments are undertaken on the Lipton ® tea the "extractable fraction" is comparable to the sum of NPE, AS, and AI fractions quantified by Keuskamp et al. (2013) using the chemical extraction method (Table 1), again allowing results from this study to be directly compared into the wider TBI literature.

Both the green and Rooibos tea have a higher fraction of extractable materials. As the methodology used in this study cannot differentiate between the different extractable fractions (i.e., NPE, AS, AI) we cannot determine how this increased quantity of extractable material may impact the recalcitrance of the tea OM. Additionally, the Rooibos tea used in this study does differ from that of Keuskamp et al. (2013); it has a significantly lower concentration of N, which in turn results in a high C/N ratio. While there are differences

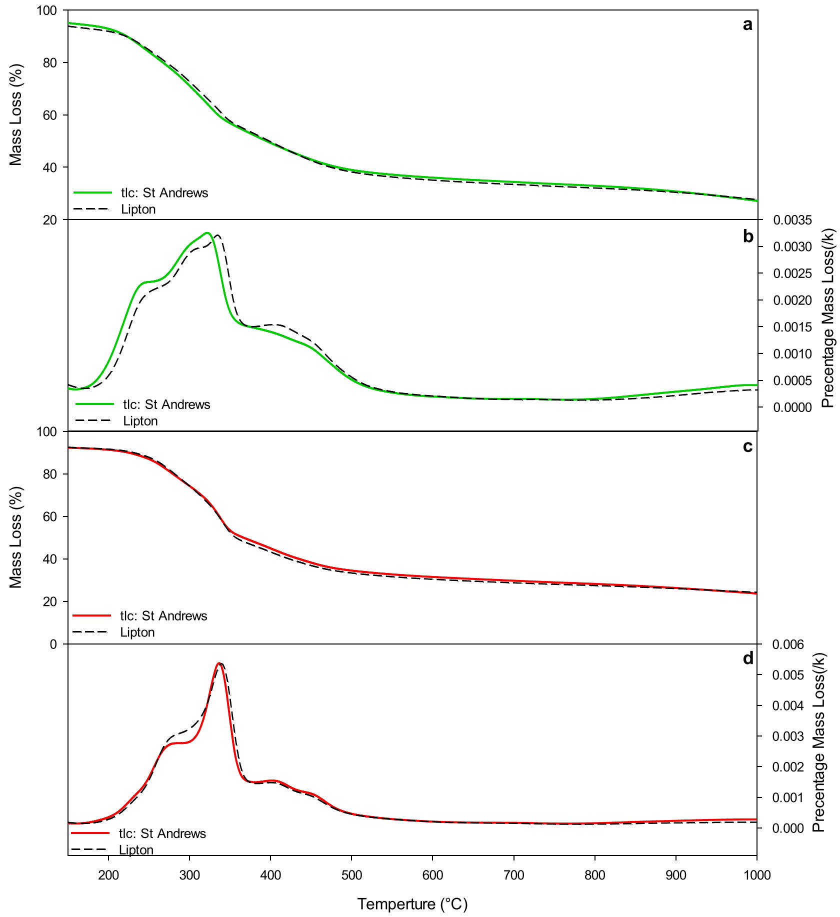

Figure 2. Comparison of the tlc: St Andrews and Lipton ® teas using thermogravimetric analyses. (a) Normalized mass loss for the green teas. (b) Percentage mass loss per degree Kelvin for the green teas. (c) Normalized mass loss for the Rooibos teas. (d) Percentage mass loss per degree Kelvin for the Rooibos teas.

between the tea used in this study to that of standardized Lipton® tea, we believe these to have minimal impact on the experiment and are confident that the results from this study are broadly comparable to other teabag incubation studies (Djukic et al., 2018; Keuskamp et al., 2013; Pouyat et al., 2017).

Table 1
Quality Parameters and Weights of the Green and Rooibos Tea Utilized in This Study (n = 5) Compared to the Teabags Used in Keuskamp et al. (2013)

	Green tea		Rooibos tea	
	This study	Keuskamp et al. (2013)	This study	Keuskamp et al. (2013)
Total mass (g)	2.433 ± 0.12	2·019 ± 0·026	2.194 ± 0.35	2·152 ± 0·013
Empty bag mass (g)	0.264 ± 0.002	0.246 ± 0.001	0.265 ± 0.001	0.245 ± 0.001
Tea mass (g)	2.169 ± 0.118	1.773 ± 0.025	1.929 ± 0.349	1.907 ± 0.025
Water-soluble fraction (g/g)	0.411 ± 0.06	0.493 ± 0.021	0.186 ± 0.02	0.215 ± 0.009
Nonpolar extractable fraction (g/g)		0.066 ± 0.003		0.049 ± 0.013
Acid soluble fraction (g/g)		0.283 ± 0.017		0.289 ± 0.040
Acid insoluble fraction (g/g)		0.156 ± 0.009		0.444 ± 0.040
Extractable fraction (g/g)	0.588 ± 0.06	0.505 ± 0.019[a]	0.881 ± 0.02	0.782 ± 0.058[a]
Mineral fraction (g/g)	0.001 ± 0.0002	0.002 ± 0.0009	0.003 ± 0.0007	0.004 ± 0.0006
OC (%)	47.26 ± 0.81	49.06 ± 0.11	47.55 ± 0.61	50.51 ± 0.29
N (%)	4.23 ± 0.07	4.02 ± 0. 05	0.64 ± 0.05	1.19 ± 0.05
C/N ratio	14.0 ± 1.9	12.2 ± 0.13	77.92 ± 16.1	42.8 ± 1.84

[a]The total was calculated from the sum of the nonpolar extractable, acid soluble, and acid insoluble fractions.

4.1.1. Incubation Experiment

Leaching experiments (Figure 3) revealed that green tea is the most soluble in water, with a total mass loss of 41.11% ± 0.6 in 36 hr with 97% of that mass loss occurring within 24 hr. In comparison, the Rooibos tea lost a total of 18.6% ± 0.2 of its original mass with 91% of that loss occurring within 24 hr. The total mass losses in this study are similar to those found by Keuskamp et al. (2013), Pouyat et al. (2017), and Djukic et al. (2018), but unlike these studies, we exclusively focus on an intertidal environment which is flushed twice daily with salt water. Therefore, the loss rate of the water-soluble fraction is likely to be a critical factor in intertidal settings as these systems are flushed twice daily with brackish/saline waters saturating the soils and leaching the water-soluble fraction of the tea.

Previous laboratory-based incubations (Djukic et al., 2018; Keuskamp et al., 2013; Pouyat et al., 2017) have purely focused on mass loss, but since the ultimate goal of these teabag studies is to understand the preservation of OC, we also measured the OC changes associated with the loss of the water-soluble fraction. The OC content of the green tea decreased by 17.55% ± 2.7 and by 3.67% ± 2.3 in the Rooibos over the 36-hr incubation period of the experiment. However, there is no direct link between the total mass loss and reduction in OC content (Figure S9). We find that the Rooibos tea displays a weak correlation ($R^2 = 0.41$) between mass and OC loss, while the green tea displays no correlation ($R^2 = 0.13$).

4.2. Soil Characteristics

The back-barrier and estuarine saltmarsh soils show distinct differences in their geochemical and physical properties (Figure 4). The back-barrier marsh soils (Sites 1-7) are characterized by a coarse (sand rich) matrix with low OC contents ranging between 0.4% and 1.3%. The estuarine marsh soils (Sites 9-12) in comparison have significantly higher OC contents (6% - 7.3%) and are fine grained (silt and clay rich) in nature (Figure 4). Although the intertidal flat (Site 8) has similar OC and inorganic carbon contents to the back-barrier marsh, the C/N and $\delta^{13}C_{org}$ values suggest the source of the OM held in the intertidal flat in similar to that found in the estuarine marsh.

By comparing the C/N ratios and $\delta^{13}C_{org}$ values of the saltmarsh soils to known Scottish values (Smeaton & Austin, 2017; Thornton et al., 2015), we find that the sources of OC to these marshes differ. The back-barrier marsh is characterized by high C/N ratios and depleted $\delta^{13}C_{org}$ values suggesting that the OC mainly originates from in situ C3 production or the terrestrial environment. In comparison, the intertidal flat and estuarine marsh soils have lower C/N ratios and enriched $\delta^{13}C_{org}$ values, which potentially indicates that the OC comes from multiple sources with a more significant marine (allochthonous) input contributing to the estuarine marsh soil.

4.3. TBI

4.3.1. OM Decomposition

The decomposition of fresh litter normally occurs in three phases (Valiela et al., 1985) as shown in Figure 5a. The first phase occurs quickly, with the water-soluble fraction (sugars and starch, etc.) being lost through leaching and rapid microbial assimilation. At all three sites, we observed significant reductions in mass, which are attributed to the leaching of the water-soluble fraction (Figures 5b–5d). Using

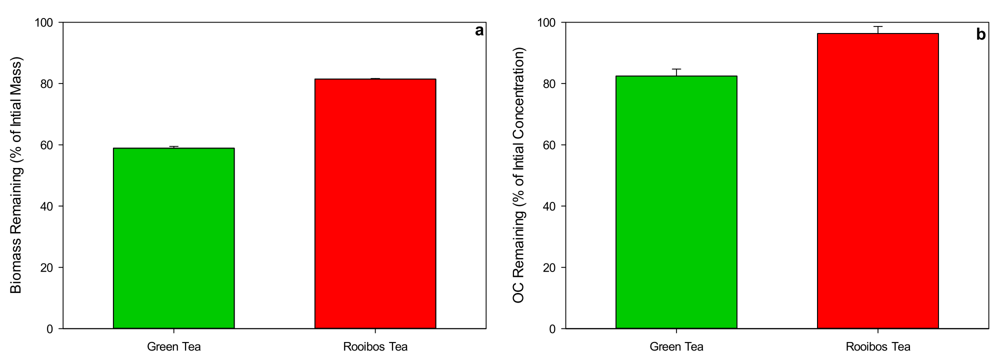

Figure 3. Results of (36 hr) incubation experiment detailing (a) average remaining mass of tea and (b) average organic carbon (OC) remaining of green and Rooibos tea after soaking.

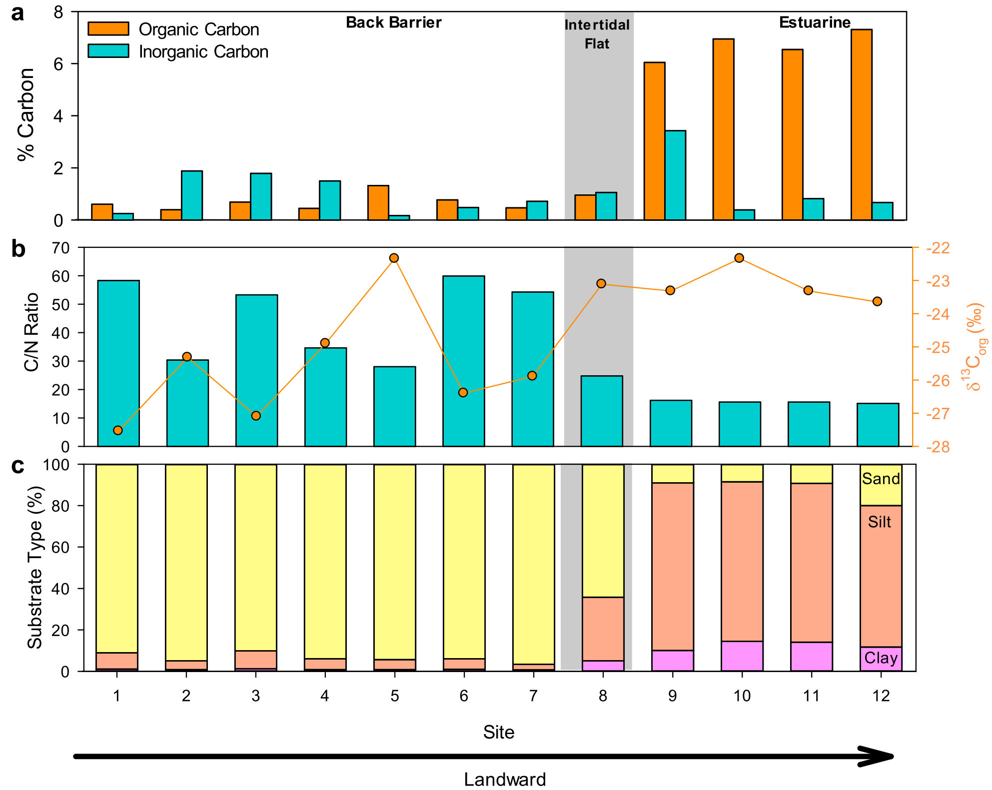

Figure 4. Characterization of the saltmarsh soils at the teabag burial depth. (a) Organic and inorganic carbon contents, (b) C/N ratios and $\delta^{13}C_{org}$ (‰ VPDB). (c) Sediment substrate classification, clay (<4 μm); silt (4–63 μm), and sand (>63 μm). VPDB = Vienna Peedee belemnite.

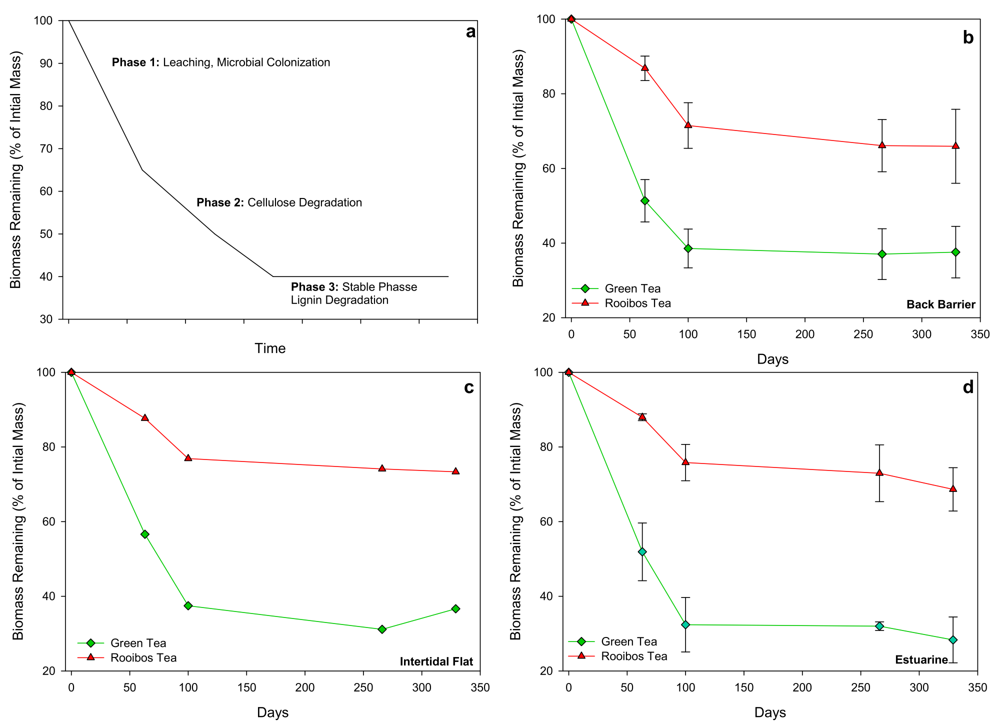

Figure 5. Green and Rooibos tea decomposition across collections. (a) Conceptual model of litter decomposition adapted from Valiela et al. (1985). Biomass reaming at the (b) back-barrier, (c) intertidal flat, and (d) estuarine sites; after 63-, 100-, 266-, and 329-day field incubation.

our understanding of the water-soluble fraction developed from the laboratory incubation experiments (section 4.1.1), we estimate that 93–99% of the green tea mass loss and 100% of the Rooibos tea mass loss between the burial and the 63-day collection was due to the leaching of the water-soluble fraction. The laboratory leaching experiment clearly showed that the initial mass loss can be rapid (within 24 hr) yet due to the collection schedule, we are unable to fully constrain the impact and the rate at which leaching occurs in the natural environment.

The second phase of decomposition involves the loss of structural components (cellulose), and was inferred from the mass loss observed at all three sites between the 63- and 266-day collection. By excluding the decrease in mass due to the inferred leaching phase of the water-soluble fraction, we can estimate the mass loss due to the initial stages of the second phase of decomposition (Table 2). Across all sites, we estimate that between 21% and 30% of the total mass loss of the green tea is due to the loss of these structural components. Across these sampled habitats, the estuarine marsh samples lost the greatest mass during this decomposition phase, followed by the intertidal flat and back-barrier marsh samples. The Rooibos tea lost significantly less mass during this second phase of decomposition, with an estimated decrease of 8–16% of the original mass.

The final phase of fresh litter decomposition is described as the metastable phase (Valiela et al., 1985), where there is a slow decrease in mass that occurs due to the gradual loss of lignin, Valiela et al. (1985) reports that this phase of decomposition takes place over a 2 year period. Neither the intertidal flat nor the back-barrier marsh show mass losses that suggests a transition into this phase of decomposition. Though a reduction in mass is observed during the final collection at the estuarine marsh (Figure 5d), it is highly likely that this is due to further cellulose (i.e., second phase) decomposition, rather than a transition to the final stage of litter decomposition

Each habitat shows distinct differences in the decomposition rate during the yearlong field incubation (Figure 5). Of the two marsh environments, the estuarine site lost the greatest mass of green tea (71.6% ± 6.1) in comparison to the back barrier which lost 62.4% ± 6.8. In contrast, the Rooibos tea shows a decrease in mass of 31.4% ± 5.8 compared to 34% ± 9.9 from the back-barrier. When these decomposition values are compared to the global synthesis produced by Djukic et al. (2018), both the intertidal flat and estuarine marsh are largely comparable with results from temperate climates (Figure 6). Despite comparable mass loss results from temperate and warm temperate climates (Djukic et al., 2018), the observations from the Tyne estuary are more variable and the back-barrier marsh results fall outside what would be expected from a temperate region. The variability in these results is likely a reflection of the different incubation periods, and within this study there are large variations observed, notably in biomass loss between the 63- and 100-day collections (Figure 6).

4.3.2. Elemental Composition

An overlooked aspect of the global TBI experiments has been an evaluation of the elemental composition of the tea and how this changes during decomposition. Of the three settings, green tea in the estuarine marsh has an OC loss pattern similar to that of biomass loss (Figure 5). In comparison, the rate at which tea OC is lost in the back-barrier marsh is significantly lower, with the OC content of the green tea reducing by less than 5% over the incubation, while the OC content of the Rooibos remains stable, a pattern also observed in the estuarine setting. In contrast both the green and Rooibos tea buried in the intertidal flats lose a significant quantity of OC (Figure 7). When compared, the mass and OC loss data do not correlate and show no covariance over the incubation period (Figure 7). However, both the N and the C/N ratios of the biomass show covariance with mass loss associated with decomposition (Figure 8).

Table 2
Total and Nonleached Biomass Loss for Both Green and Rooibos Tea From the Different Habitats

		Biomass loss (% of initial mass)					
		Back barrier		Mudflat		Estuarine	
	Water-soluble fraction (%)	Total	Nonleached	Total	Nonleached	Total	Nonleached
Green tea	41.4 ± 0.6	62.4 ± 6.8	21.0 ± 6.8	63.4	22.0	71.6 ± 6.1	30.2 ± 6.1
Rooibos tea	18.5 ± 0.2	34.0 ± 9.9	15.5 ± 9.9	26.6	8.1	31.4 ± 5.8	12.9 ± 5.8

The N content of both teas changed significantly during the incubation period (Figure 9). The relative N content increased after the 63- and 100-day collections (266-day collection for the Rooibos tea at the intertidal flat sampling site). N immobilization (i.e., locked away by microorganisms and unavailable to plants) is potentially a mechanism that could facilitate the changes observed. Currently our understanding of N immobilization in intertidal environments is relatively underdeveloped in comparison to terrestrial examples (e.g., Berg & McClaugherty, 1987). N immobilization has been observed in Californian saltmarshes where the main pathway for the process was nitrate retention via microbial uptake which outpaced N losses through denitrification (Yang et al., 2015). After the 100-day collection, we observed a slow decrease in N content, which suggests a transition to N release and mineralization. The release of N increases at the final stages of the incubation experiment (Figure 9). It appears that N immobilization from the green and Rooibos tea differ in the intertidal flat, with peak N immobilization inferred at the time of the 100- and 266-day collections for the green and Rooibos teas, respectively (Figure 9). There is also an indication of N mineralization taking place by the time of the final collection (329 days). Minimal OC loss combined with N immobilization acts to drive down the C/N ratios of the OM. During the early stage of decomposition, N is rapidly immobilized and the C/N of both teas across all habitats decreases; the impact is most pronounced in the C/N ratio of the more bioavailable green tea because its rate of initial C loss is the most rapid.

5. Discussion

Climate is considered one of the main drivers of litter decomposition in multiple recent studies (Djukic et al., 2018, Mueller et al., 2018). However, the Tyne estuary experiment shows that even under the same climatic and broad environmental conditions, there is significant variability in the rate and magnitude of biomass degradation and therefore in the potential for OC storage in marshes within the same climate regime. Our results suggest that 93–100% of the tea mass loss recorded in the first 3 months is probably due to the loss of the water-soluble fraction (Figure 5). It seems highly likely that this water-soluble fraction was rapidly lost after burial due to in situ environmental conditions (i.e., twice daily tidal inundation driving the initial biomass loss: Djukic et al., 2018, Mueller et al., 2018).

By extending the incubation period beyond 3 months, we were able to observe the initial stages of decomposition of the nonleachable fraction (i.e., cellulose). The degradation of this fraction differs between the environments studied, suggesting that climate and tidal inundation are not the main drivers which govern the breakdown of these more recalcitrant OM components. Rather, the main controls on long-term decomposition of fresh OM are the soil type and presumably the overall preservation conditions (e.g., oxygen availability, microbial activity). These effects are demonstrated by a comparison of the results from the two Tyne estuary marshes (Figure 5). The back-barrier marsh environment preserved the greatest quantity of both green and Rooibos tea, potentially due to the low oxygen conditions and/or reduced microbial activity within the soil, thereby inhibiting the OM breakdown despite the soil itself being largely a porous sand. In contrast, the teabags buried in the muddy estuarine marsh underwent an initial mass loss (3 months) equivalent to that of the back-barrier and intertidal flat. These results suggest that an interplay between climate (temperature, precipitation) and environmental conditions (tidal cycle) drive the initial decrease in mass, but subsequent OM decomposition potentially depends more on soil type and the preservation conditions, warranting greater focus in future TBI studies. Therefore, we would strongly recommend that leaching be taken into account in estimates of biomass loss within these intertidal systems, with the possibility of applying a leaching correction as outlined by Seelen et al. (2019). Furthermore, we also recommend that all future TBI experiments are carried out beyond 3 months, ideally to a minimum of 1 year to capture the initiation of cellulose decomposition taking place in these environments. As this study did not fully resolve the

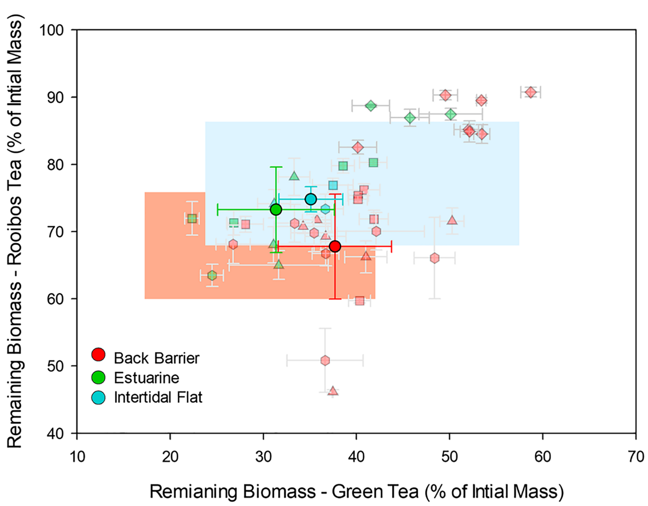

Figure 6. Remaining mass of tea litter (expressed as a percent of initial mass) of different types (green and Rooibos tea) after yearlong incubation. The shaded boxes represent the global synthesis of data from similar studies in regions of temperate (blue), warm temperate (orange) climate (Djukic et al., 2018). The different symbols represent the different collection times (■) *Collection 1–100 days*, (●) *Collection 2–266 days*, (▲) *Collection 3–329 days.* (◆)*Collection 4–63 days* is displayed in the plot but not included in the mean calculations.

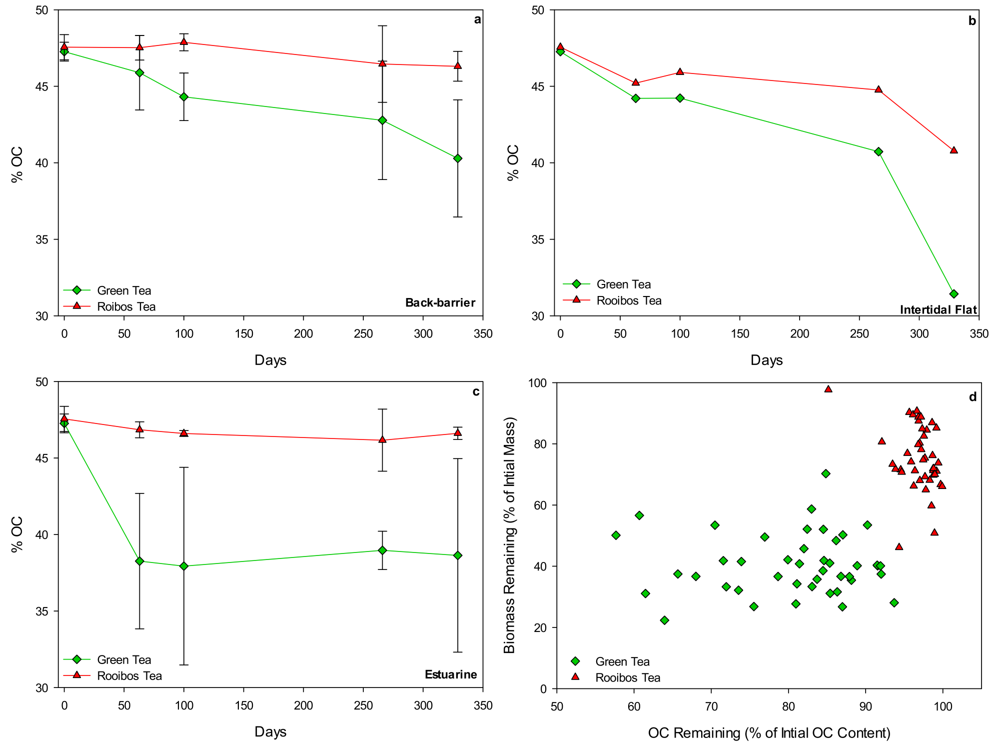

Figure 7. Organic carbon content remaining from the teabag incubations in (a) the back-barrier marsh, (b) intertidal flat, and (**c**) estuarine marsh. (**d**) Organic carbon loss versus biomass loss (green: $R^2 = 0.000002$, Red: $R^2 = 0.1$).

second phase of and misses the final phases of decomposition outlined by Valiela et al. (1985), we acknowledge that it is advisable to extend the incubation period to >2 years.

One of the goals of TBI studies should be to inform an improved understanding of the early degradation and potential for OC storage in underlying soils. This is also a goal for intertidal blue carbon environments, such as saltmarshes; we therefore set out to understand how the elemental composition of buried tea, which has not been previously reported, changed over the incubation period. When the tea biomass and OC loss results from both the field (Figure 7) and laboratory (Figure S9) incubations are compared, there is no indication of a correlation between OM and OC. This apparent disconnect between OC and biomass loss over the incubation period might, initially, seem counterintuitive because of the expectation that OC content decreases alongside biomass loss over the yearlong incubation period. However, since OM is not solely OC, but comprises a number of additional elements, particularly N and phosphorus (P), this need not be the case. The loss of these

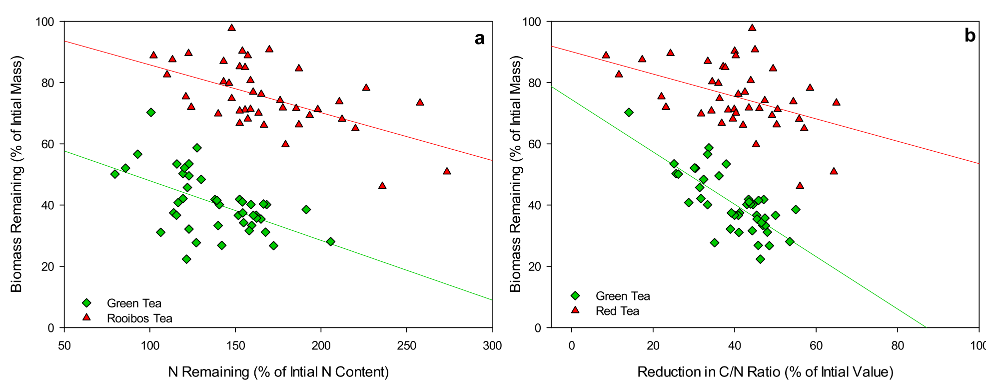

Figure 8. Biomass remaining from all teabag collections compared to (a) N remaining (green: $R^2 = 0.29$, red: $R^2 = 0.32$) and (b) reduction in C/N ratio (green: $R^2 = 0.56$, red: $R^2 = 0.2$).

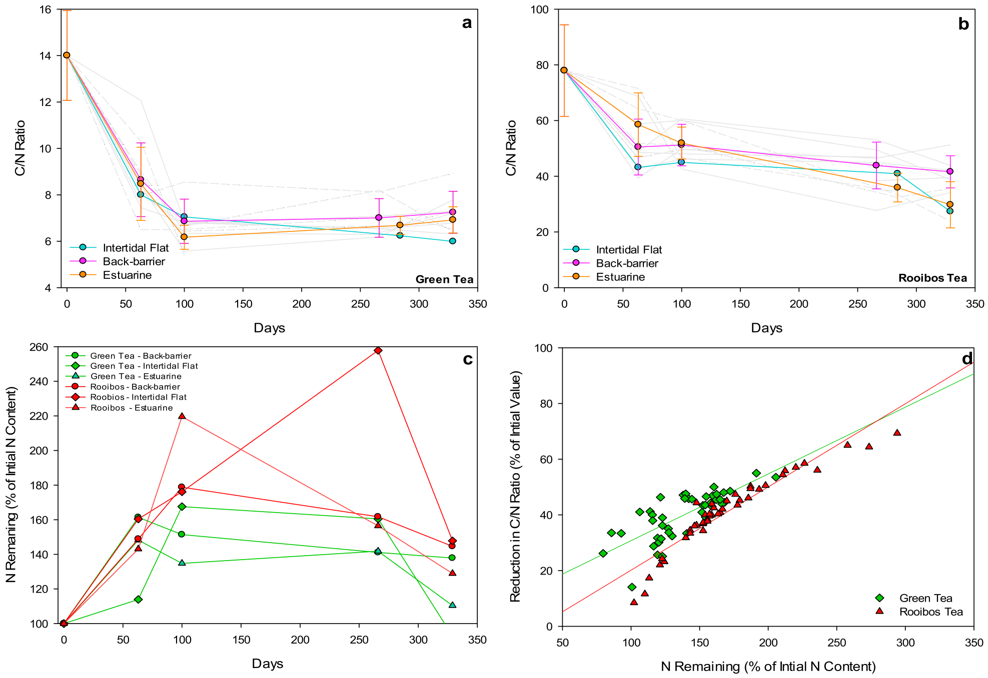

Figure 9. Nitrogen and C/N ratio over the incubation experiment after 63-, 100-, 266-, and 329-day field incubation. (a) C/N ratios of the green tea during the incubation. (b) C/N ratios of the Rooibos tea during the incubation. (c) N immobilization during the incubation. (d) Comparison of changes in C/N ratio versus N for the green tea ($R^2 = 0.59$) and Rooibos tea ($R^2 = 0.89$).

elements would decrease the overall biomass of the litter without necessarily altering the OC content; this process has been observed in natural intertidal systems (Fourqurean & Schrlau, 2003; Valiela et al., 1985).

While we do not have a full understanding of the elemental stoichiometry of the two types of tea, we can make inferences using the bulk elemental data (Table 1). The green and Roobios tea have OC contents of 47.26% and 47.55% respectively; therefore, >50% of the biomass is made up of different elements and there is no reason to expect a simple 1:1 relationship between biomass loss and OC content. Furthermore, this disconnect is likely to be exaggerated by the different soil characteristics at each incubation site (Figure 4). However, using the biomass loss data which has been corrected for leaching (Table 2) in conjunction with the elemental data, (Table 1) there is potential to create a simple correction to qualitatively estimate the quantity of OC lost during the incubation. By adjusting the percent biomass loss (nonleached) by the known OC content (i.e., green: 47.26 % and Rooibos: 47.55 %) we can predict how much OC could be lost during the incubation period (Figure 10). In comparison with direct field measurements, this method overestimates the OC loss from the Rooibos tea; this is probably due to the more recalcitrant nature of the Rooibos tea. This approach broadly allows for the OC lost from the green tea to be predicted from biomass loss data at both the back-barrier and estuarine sites. The intertidal flats differ, as the measured OC loss is greater than the predicted value (Figure 10). When the biomass loss (Figure 5) and OC loss (Figure 7) rates are compared, there is a disconnect with noticeable OC loss occurring at the end of the incubation with no corresponding biomass loss. This is potentially due to the lack of replicate samples across the intertidal flat or possibly due to natural processes as this environment is the most dynamic of the three sites with biofilm growth, shifting mud and channels alongside diverse OC sources.

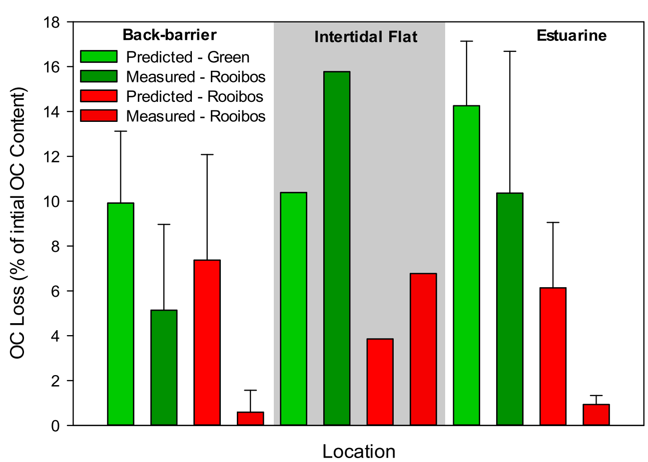

Figure 10. Observed organic carbon (OC) loss versus predicted OC loss for the three environmental settings for both the green and Rooibos tea over the 1-year incubation.

The results from this study may differ from other tea bag studies because the elemental abundances, notably in the Rooibos tea, differ from the standard Lipton® tea. This study has highlighted the need for the wider TBI community to move beyond the original Lipton® brand teas to different brands. The original Lipton® tea bags used by Keuskamp et al. (2013) are becoming scarce, requiring other brands to be considered and comparative studies to be undertaken to assure the continuity of future TBI studies to that of the past. This study has taken the first steps toward this goal but a concerted effort from the global TBI community is required to assure the TBI remains a usable tool for comparing different environments.

However, the differences in tea brands does not detract from the observation that OM and OC losses proceed at different rates and we would argue that greater consideration should therefore be given to the application of the TBI as a proxy for OC degradation in intertidal environments. Going forward, a more comprehensive understanding of the elemental stoichiometry would allow the TBI to be used as an improved proxy in OC degradation studies.

While the TBI method does provide data on plant litter decomposition which can be used to infer the rate at which plant OC degrades, it must be remembered that fresh plant litter is only one competent of the OM input into intertidal settings. The OC accumulating in these habitats is comprised of varying amounts of fresh litter and allochthonous terrestrial and marine components, which in turn degrade over significantly different timescales. As the TBI is now employed globally by over 1,200 groups (Ogden, 2017), our results highlight the need for caution in the application of TBI as a universal metric for the breakdown of these organic components within saltmarsh habitats.

6. Conclusion

Our results indicate that two major assumptions of TBI studies, namely that decomposition is controlled by climate and that mass loss of tea can be used as a proxy for OC loss, may not apply across all intertidal environments. We find that sediment type and presumably variables including oxygen availability and microbial activity are a major control on long-term OM storage potential, with important implications for management decisions to preserve C rich saltmarsh ecosystems (e.g., Kelleway et al., 2017). Furthermore, significant changes in the tea biomass were only observed after an incubation period of 1 year, yet it is unclear if we captured the full extent of this process. This suggests that short-term (90 day) incubations are of insufficient duration and that burial periods of >1 year are required to understand the OM degradation processes in intertidal environments. TBI studies in intertidal environments should be treated with caution when applying litter mass loss as a proxy for plant litter OC loss within the first year of decomposition. Despite the need for caution, the TBI method remains a useful and cost-effective tool in understanding and comparing broad decomposition dynamics of fresh litter within and between intertidal environments.

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